LISTING OF CLAIMS

The following listing of claims replaces all prior versions:

1. (Currently amended) A method for the directional subcloning of DNA fragments comprising:

a) providing a first vector comprising a first selectable marker gene and a DNA sequence of interest having an open reading frame, which DNA sequence of interest is flanked by at least two restriction enzyme sites, wherein at least one of the flanking restriction enzyme sites is a site for a first restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates complementary single-strand DNA 3' TA overhangs, wherein at least one of the flanking restriction enzyme sites is for a second restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends ~~that are not complementary to the overhangs generated by the first restriction enzyme~~, wherein digestion of the first vector with the first restriction enzyme and the second restriction enzyme generates a first linear DNA fragment which lacks the first selectable marker gene but comprises the DNA sequence of interest~~, wherein the first restriction enzyme generates a 3' TA overhang~~;

b) providing a second vector comprising a second selectable marker gene which is distinguishable from the first selectable marker gene, and ~~non-essential~~ a DNA sequence[s,] ~~optionally including~~ encoding a ~~counterselectable~~ lethal gene[,] ~~which non-essential sequences are~~ flanked by at least two restriction enzymes sites, wherein at least one of the flanking restriction enzyme sites in the second vector is for a third restriction enzyme which generates complementary single-strand DNA overhangs that are complementary to the single-strand DNA overhang generated by the first restriction enzyme in the first linear DNA fragment, wherein at least one of the flanking restriction sites in the second vector is for a fourth restriction enzyme which generates blunt ends ~~that are not complementary to the ends generated by the first or third restriction enzyme but can be ligated to the ends generated by the second restriction enzyme~~, wherein digestion of the second vector with the third restriction enzyme and the fourth restriction enzyme generates a second linear DNA fragment which lacks the lethal gene ~~non-essential DNA sequences~~ but comprises the second selectable marker~~, which second linear DNA fragment is flanked by~~ and having ends which permit the oriented joining of the first linear DNA fragment to the second linear DNA fragment, ~~wherein the second and fourth restriction enzymes generate blunt ends,~~ wherein sequences including a portion of the overhang generated by the third restriction enzyme include one or more codons that after oriented joining are in-frame with the open reading frame, thereby encoding a N-terminal fusion with the gene product encoded by the open reading frame and/or wherein sequences forming a portion of and 3' to the cleavage site generated by the fourth restriction enzyme include one or more nucleotides for a stop codon that after oriented joining are in-frame with the open reading frame or for one or more codons that after oriented joining are in-frame with the open reading frame, thereby encoding a C-terminal fusion with the gene product encoded by the open reading frame; and

c) combining the first and second vectors, the first vector and the second linear DNA fragment, or the second vector and the first linear DNA fragment in a suitable buffer with one or more restriction enzymes and optionally DNA ligase under conditions effective to result in digestion and ligation to yield a mixture comprising a third vector comprising the first and second linear DNA molecules which are joined in an oriented manner.

2. (Cancelled).

3. (Original) The method of claim 1 wherein the first and third restriction enzymes are not the same.

4. (Original) The method of claim 1 wherein the second and fourth restriction enzymes are not the same.

5. (Cancelled).

6. (Original) The method of claim 1 wherein the first restriction enzyme is *Sgf*I.

7. (Original) The method of claim 6 wherein the second restriction enzyme is *Pme*I.

8. (Cancelled).

9. (Previously Presented) The method of claim 1 wherein the third restriction enzyme is *Pvu*I or *Pac*I.

10. (Original) The method of claim 1 wherein the DNA sequence of interest comprises an open reading frame comprising one or more sites for the first or second restriction enzyme.

11. (Original) The method of claim 10 wherein prior to digestion with the one or more restriction enzymes, the sites for the one or more restriction enzymes in the open reading frame are protected so as to prevent digestion.

12. (Original) The method of claim 11 wherein the sites are protected by methylation.

13. (Original) The method of claim 12 wherein prior to methylation the flanking sites for the first or second restriction enzyme are contacted with an oligonucleotide complementary to the flanking restriction enzyme site and RecA.

14-35. (Cancelled).

36. (Previously presented) The method of claim 1 wherein one of the restriction enzymes is *Dra*I, *Nru*I, *Pac*I, *Pme*I, *Pvu*I, *Sgf*I, *Srf*I, or *Swa*I, or a restriction enzyme that has the same recognition site as *Dra*I, *Nru*I, *Pac*I, *Pme*I, *Pvu*I, *Sgf*I, *Srf*I, or *Swa*I.

37-83. (Cancelled).

84. (Previously presented) The method of claim 1 wherein the first restriction enzyme is *Sgf*I and the second restriction enzyme is *Pme*I.

85. (Previously presented) A method for the directional subcloning of DNA fragments comprising:

a) providing a first vector comprising a first selectable marker gene and a DNA sequence of interest having an open reading frame, which DNA sequence of interest is flanked by at least two restriction enzyme sites, wherein at least one of the flanking restriction enzyme sites is a site for a first restriction enzyme that generates complementary single-strand DNA overhangs, wherein at least one of the flanking restriction enzyme sites is for a second restriction enzyme that generates blunt ends ~~that are not complementary to the overhangs generated by the first restriction enzyme~~, wherein digestion of the first vector with the first restriction enzyme and the second restriction enzyme generates a first linear DNA fragment which lacks the first selectable marker gene but comprises the DNA sequence of interest;

b) providing a second vector comprising a second selectable marker gene which is distinguishable from the first selectable marker gene, and ~~non-essential~~ a DNA sequence[s,] ~~optionally including~~ encoding a ~~counterselectable~~ lethal gene[,] ~~which non-essential sequences are~~ flanked by at least two restriction enzymes sites, wherein at least one of the flanking restriction enzyme sites in the second vector is for a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates complementary single-strand 3'TA DNA overhangs that are complementary to the single-strand DNA overhang generated by the first restriction enzyme in the first linear DNA fragment, wherein at least one of the flanking restriction sites in the second vector is for a fourth restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends ~~that are not complementary to the ends generated by the first or third restriction enzyme but can be ligated to the ends generated by the second restriction enzyme~~, and wherein digestion of the second vector with the third restriction enzyme and the fourth restriction enzyme generates a second linear DNA fragment which lacks the lethal gene ~~non-essential DNA sequences~~ but comprises the second selectable marker~~, which second linear DNA fragment is flanked by~~ and having ends which permit the oriented joining of the first linear DNA fragment to the second linear DNA fragment, ~~wherein the second and fourth restriction enzymes generate blunt ends, wherein the third restriction enzyme generates a 3' TA overhang,~~ wherein sequences including a portion of the overhang generated by the third restriction enzyme include one or more codons that after oriented joining are in-frame with the open reading frame, thereby encoding a N-terminal fusion with the gene product encoded by the open reading frame and/or wherein sequences forming a portion of and 3' to the cleavage site generated by the fourth restriction enzyme include one or more nucleotides for a stop codon that after oriented joining are in-frame with the open reading frame or for one or more codons that after oriented joining are in-frame with the open reading frame, thereby encoding a C-terminal fusion with the gene product encoded by the open reading frame; and

c) combining the first and second vectors, the first vector and the second linear DNA fragment, or the second vector and the first linear DNA fragment in a suitable buffer with one or more restriction enzymes and optionally DNA ligase under conditions effective to result in digestion and optionally ligation to yield a mixture comprising a third vector comprising the first and second linear DNA molecules which are joined in an oriented manner.

86. (Cancelled)

87. (Previously presented) The method of claim 85 wherein the first and third restriction enzymes are not the same.

88. (Previously presented) The method of claim 85 wherein the second and fourth restriction enzymes are not the same.

89. (Previously presented) The method of claim 85 wherein the third restriction enzyme is *Sgf*I.

90. (Previously presented) The method of claim 89 wherein the fourth restriction enzyme is *Pme*I.

91. (Previously presented) The method of claim 85 wherein the first restriction enzyme is *Pvu*I or *Pac*I.

92. (Previously presented) The method of claim 85 wherein the first restriction enzyme is *Sgf*I and the second restriction enzyme is *Pme*I.

93. (Previously presented) The method of claim 1 or 85 wherein the sequences including a portion of the overhang generated by the third restriction enzyme include one or more codons that after oriented joining are in-frame with the open reading frame.

94. (Previously presented) The method of claim 93 wherein the sequences forming a portion of and 3' to the cleavage site generated by the fourth restriction enzyme include one or more nucleotides for the stop codon.

95. (Previously presented) The method of claim 93 wherein the sequences forming a portion of and 3' to the cleavage site generated by the fourth restriction enzyme include the one or more nucleotides for the one or more codons that after oriented joining are in-frame with the open reading frame.

96. (Previously presented) The method of claim 1 or 85 wherein the sequences forming a portion of and 3' to the cleavage site generated by the fourth restriction enzyme include the one

or more nucleotides for the one or more codons that after oriented joining are in-frame with the open reading frame.

97. (Previously presented) The method of claim 96 wherein the open reading frame contains an in-frame initiation codon.

98. (Previously presented) The method of claim 1 or 85 wherein the open reading frame includes an in-frame initiation codon and an in-frame stop codon.

99. (Previously presented) The method of claim 1 or 85 wherein one of the vectors further comprises a site for *Aar*I, *Asc*I, *Bbr*CI, *Csp*I, *Fse*I, *Not*I, *Sap*I, *Sda*I, *Sfi*I, or *Spl*I, or a restriction enzyme that has the same recognition site as *Aar*I, *Asc*I, *Bbr*CI, *Csp*I, *Fse*I, *Not*I, *Sap*I, *Sda*I, *Sfi*I, or *Spl*I.

100. (Previously presented) The method of claim 1 or 85 wherein the second vector comprises a promoter which is 5' to the recognition site for the third restriction enzyme and positioned so that an open reading frame, introduced by ligating the first linear DNA fragment with the second linear DNA, is capable of being transcribed from the promoter.

101. (Previously presented) The method of claim 1 or 85 wherein the sequences including a portion of the overhang generated by the third restriction enzyme include one or more codons that after oriented joining are in-frame with the open reading frame and wherein the sequences forming a portion of and 3' to the cleavage site generated by the fourth restriction enzyme include one or more nucleotides for a stop codon that after oriented joining are in-frame with the open reading frame or for one or more codons that after oriented joining are in-frame with the open reading frame, thereby encoding a N-terminal fusion and a C-terminal fusion with the gene product encoded by the open reading frame.

102. (Previously presented) The method of claim 93 wherein the third vector encodes a N-terminal fusion but not a C-terminal fusion.

103. (Previously presented) The method of claim 102 wherein the first or third restriction enzyme is *Sgf*I.

104. (Previously presented) The method of claim 102 wherein the second restriction enzyme is *Pme*I.

105. (Previously presented) The method of claim 96 wherein the third vector encodes a C-terminal fusion but not a N-terminal fusion.

106. (Previously presented) The method of claim 105 wherein the first or third restriction enzyme is *Sgf*I.

107. (Previously presented) The method of claim 105 wherein the second restriction enzyme is *Pme*I.

108. (Previously presented) The method of claim 101 wherein the first or third restriction enzyme is *Sgf*I.

109. (Previously presented) The method of claim 101 wherein the second restriction enzyme is *Pme*I.

110. (New) The method of claim 1, wherein the lethal gene encodes a barnase.

111. (New) The method of claim 85, wherein the lethal gene encodes a barnase.